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Endocrinology of sociality: comparisons between sociable and solitary individuals within the same population of African striped mice

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Abstract: The social organization of species ranges from solitary-living to complex social groups. While the evolutionary reasons of group-living are well studied, the physiological mechanisms underlying alternative social systems are poorly understood. By studying group-living and solitary individuals of the same species, we can determine hormonal correlates of sociality without the problem of confounding phylogenetic factors. The African striped mouse (*Rhabdomys pumilio*) is a socially flexible species, which can be solitary or alternatively form complex family groups, depending on population density and the extent of reproductive competition. We predicted group-living striped mice to show signs of reproductive suppression and social stress, resulting in higher corticosterone but lower testosterone levels when compared to solitary-living individuals. To determine whether differences in social organization correlated with hormonal differences, we collected blood samples from free-living striped mice during four breeding seasons when we experimentally induced solitary-living in philopatric individuals by locally reducing population density. Striped mice that were group-living did not change their corticosterone or testosterone levels during the study, indicating that there was no temporal effect during the breeding season. Striped mice of both sexes had significantly lower corticosterone levels after switching from group- to solitary-living. Solitary males – but not solitary females – had higher testosterone levels than group-living conspecifics. Our results suggest that group-living results in physiological stress and can induce reproductive suppression, at least in philopatric males. The switch to solitary-living may thus be a tactic to avoid reproductive competition within groups, and is associated with decreased stress hormone levels and onset of independent reproduction.

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3 **ABSTRACT**

4 The social organization of species ranges from solitary-living to complex social groups. While the
5 evolutionary reasons of group-living are well studied, the physiological mechanisms underlying
6 alternative social systems are poorly understood. By studying group-living and solitary
7 individuals of the same species, we can determine hormonal correlates of sociality without the
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16 density. Striped mice that were group-living did not change their corticosterone or testosterone
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18 Solitary striped mice of both sexes had significantly lower corticosterone levels than individuals
19 that remained group-living. Solitary males – but not solitary females – had higher testosterone
20 levels than group-living conspecifics. Our results suggest that group-living results in
21 physiological stress and can induce reproductive suppression, at least in philopatric males. The
22 switch to solitary-living may thus be a tactic to avoid reproductive competition within groups, and
23 is associated with decreased stress hormone levels and onset of independent reproduction.

Keywords: aggression; dispersal; social flexibility; philopatry; communal breeding; cooperative breeding; alternative reproductive tactics; helper.

INTRODUCTION

Animals show a variety of different social organizations, ranging from species that live solitarily to species that form complex social groups (Wilson, 2000). Solitary and group-living species display distinct mating systems, which are believed to arise as a consequence of differences in individuals' social interactions with their conspecifics (Blumstein et al., 2010). The ecological and evolutionary reasons that cause individuals to form and maintain social groups have been extensively studied (Koenig et al., 1992; Emlen, 1995; Cahan et al., 2002), while the reasons of solitary-living have received less attention (Schradin et al., 2012b). More recently, it has been shown that relaxed ecological constraints (low population density) and high reproductive competition within groups favor solitary-living (Schradin et al., 2010; Schoepf and Schradin, 2012a), supporting previous findings (Koenig et al., 1992) and theory (Emlen, 1995). In comparison, the underlying physiological mechanisms that lead to different forms of social systems are still poorly understood.

Many studies of the proximate mechanisms of sociality have focused on the neuroendocrine system (Pfaff, 2005; Schoech et al., 2004). Hormone levels can change in a relative short period of time (Wynne-Edwards and Reburn, 2000) and, by acting directly in the brain and on the peripheral organs, play a prominent role in affecting an individual's behavior (Buntin, 1996). Glucocorticoids (e.g. corticosterone) are important modulators of stress responses as they allow individuals to react to energetically demanding situations, such as those encountered during social interactions, dispersal and

when exploring novel environments (Belthoff and Dufty, 1998; Creel, 2001; Young and Monfort, 2009). Androgens (e.g. testosterone) are strong modulators of reproduction, dominance and aggression (Moore et al., 1998; Evans et al., 2000). Most of the endocrinological research on sociality has thus far focused on comparing dominants and subordinates within the same group (Poiani and Fletcher, 1994; Carlson et al., 2004; Malueg et al., 2009) or on the role of hormones in group stability (Sapolsky, 1992) and intraspecific encounters (Marler et al., 1995). What is so far mainly missing are comparisons between solitary and group-living individuals. Solitary and group-living species are expected to differ in the way they react to environmental limitations, yet comparisons of physiological mechanisms of solitary and group-living species are very rare and difficult to interpret because of confounding phylogenetic effects (Beery et al., 2008). Studies on endocrine factors of dispersal (Nunes et al., 1999) are problematic as in most social species individuals are solitary only during the dispersal phase and do not follow a permanent solitary tactic. These problems could be avoided by studying the physiological profiles of socially flexible species.

In socially flexible species, both group-living and solitary individuals occur in the same population, and even the entire social organization of a population can switch from group- to solitary-living as a response to environmental changes (Schradin et al., 2012b). Social flexibility has been observed in several species of insects, birds and mammals where both males and females are able to change their social and reproductive tactics in response to changing environmental conditions (Schradin et al., 2012b). Studies of species with alternative reproductive tactics indicate that individuals following different tactics differ in their steroid hormone levels (Oliveira et al., 2008). Males following a

dominant bourgeois tactic also often have the highest androgen levels (Rose et al., 1971; Bartsch et al., 1992; Gould, 2005), whereas glucocorticoid patterns are not as clear because subordinate individuals of some species show high glucocorticoid levels (Creel, 2005), whereas in other species, individuals following a dominant tactic have the highest glucocorticoid levels (Creel, 2001). Nonetheless, individuals following alternative reproductive tactics differ in their hormone profile, and we can thus expect hormonal differences also between group-living and solitary individuals of the same species.

The African striped mouse (*Rhabdomys pumilio*) is a highly socially flexible species, and thus makes for an ideal model to study whether individuals that are solitary differ in their hormone profile from individuals that are group-living. When population density is high, striped mice live in extended family groups, consisting of a breeding male, two to four communally breeding females and their adult philopatric offspring of both sexes (Schradin and Pillay, 2004). Individuals of both sexes can become solitary if population density becomes low during the breeding season (Schradin et al., 2010; Schoepf and Schradin, 2012a). Striped mice of both sexes are thus able to follow one of three alternative reproductive tactics: (i) remain as non-breeding philopatrics in their natal nest; (ii) become dominant group-living breeders; or (iii) disperse and become solitary-living breeding females or solitary roaming males (Schradin and Pillay, 2003, Schradin, 2004). Corticosterone levels of non-breeding philopatric striped mice are seven times higher than those of breeding males during the breeding season, but these levels drop in the following non-breeding season (Schradin, 2008), or when males are removed from the family in captivity, indicating that philopatric males are reproductively suppressed (Schradin et al. 2009b; Schradin et al 2012b). Intra-group competition over limited

resources, such as food, can be high in group-living species (Danchin et al., 2008) and may cause additional physiological stress, which might be reduced when group size declines. Dispersal of siblings causes a decline of group size and might influence the hormone profile of those individuals remaining within the group. We predicted: a) group-living striped mice of both sexes that would later become solitary to have higher corticosterone levels than their siblings which would remain group-living (i.e. high corticosterone levels could trigger dispersal), b) corticosterone levels to decrease once individuals have dispersed and have become solitary; and c) group-living individuals to show lower corticosterone levels after group size declined due to the dispersal of other group members.

Testosterone levels of male philopatrics are lower than those of roaming and breeding males (Schradin et al., 2009a), and testosterone levels of all male and female striped mice decrease from the breeding to the non-breeding season (Schradin, 2008). Testosterone promotes sexual motivation and suppresses parental care in males (Wingfield et al., 1990; Ketterson and Nolan, 1999; Wingfield et al., 2001). Philopatric striped mice act as helpers at the nest and show high degrees of alloparental care, whereas solitary-living roaming males do not participate in parental care (Schradin et al., 2009a). Testosterone has been associated with aggression in males (Wingfield et al., 1990) and in many vertebrate species females can be as aggressive as males (Hau, 2007), including striped mice, in which individuals of both sexes are more aggressive when they become solitary than when they are group-living (Schoepf and Schradin, 2012b). In addition, testosterone is anxiolytic in laboratory mice (Aikey et al., 2002) and might promote risk-taking in roaming male striped mice (Schradin et al., 2009b). Female striped mice can

either breed communally or solitarily, with solitary-breeding likely to be the riskier tactic, since solitary breeders must find and defend a territory alone. Thus, testosterone in females might play a similar role as in males in promoting risk-taking behaviors. We predicted: a) testosterone levels of solitary individuals of both sexes to be higher than of group-living individuals; and b) testosterone levels of males and females that would later disperse to be higher than those of individuals that would remain group-living, indicating their readiness to seek independent breeding.

METHODS

Study period and field techniques

Data were collected during the breeding season (August to November) of 2007-2010 on a field site located on the farm Klein Goegap (29°42.30'S - 18°02.95'E) near the town of Springbok in South Africa. Striped mice social tactics (solitary or group-living) were determined using a combination of trapping, radio-tracking and behavioral observations (Schradin et al., 2010; Schoepf and Schradin, 2012). Striped mice were trapped at their nest, sexed, weighed and marked permanently with ear-tags (National Band and Tag Co., Newport, KY, U.S.A.) and temporarily with a non-toxic hair dye (Inecto Rapido, Pinetown, South Africa) for individual recognition during behavioral observations. All adult breeders and up to four philopatries (two females and two males) of each group were fitted with radio-collars (Holohil, Carp, Ontario, Canada; 1.2-4.5g). Striped mice were radio-tracked during the day to determine home ranges and at night to determine composition of sleeping groups, following the methods previously established by Schradin and Pillay (2005). Striped mice that spent more than 75% of the nights with

other individuals were regarded as group-living whereas those that slept alone for >75% of the nights were regarded as solitary-living; no intermediate values occurred. All mice sampled were part of a field manipulation experiment in which we tested the role of population density on sociality by removing several groups from the field site (Schoepf and Schradin, 2012a). Removal of groups was carried out in the beginning of each breeding season in the years 2007 to 2010. Altogether, 52 mice from 12 groups were removed from a 30 hectares area (for more details see Schoepf and Schradin, 2012a). Blood samples were obtained only from individuals belonging to groups that were not removed.

Collection and analysis of blood samples

Striped mice were captured directly at their nests in the early morning (between 06:15 and 8:30 depending on sunrise) within 30min after they emerged. Mice were anaesthetized with diethyl ether and blood samples were collected from a sublingual vein (Heimann, 2006). All samples were obtained within three minutes after a mouse had entered the traps to avoid a stress response (Schradin, 2008). Samples were transported to the research station, where they were left to clot at room temperature (for up to 1.5h from the time the sample was taken; ambient temperature was mainly below 20°C during the breeding season). Samples were centrifuged for 10 minutes and the obtained serum (extracted in different aliquots: 20 µl for corticosterone and 60 µl for testosterone) was stored at -20 °C. All samples were analyzed at the University of Zurich, using enzyme immuno-assays. Corticosterone and testosterone levels were measured with commercial kits from IBL Hamburg, following procedures previously validated for striped mice

(Schradin, 2008). Coefficients for intra-assay variation were 7.8% for testosterone and 11.1% for corticosterone. Coefficient for inter-assay variation was 10.2% for testosterone and 10.9% for corticosterone. 30 samples from group-living mice and 30 samples from solitary mice were analyzed for corticosterone, and 27 samples from group-living individuals and 30 samples from solitary individuals were analyzed for testosterone (Table 1). Blood samples were obtained an average of 8.55 ± 5.25 days before individuals switched from group-living to solitary and an average of 9.78 ± 5.77 days after individuals had become solitary. Six individuals (four females and two males) were re-sampled after switching from group-living to solitary. We waited 16.00 ± 3.94 days before taking blood from the same individual. All individuals used in the study were initially philopatrics, born from the first litter of the breeding season and, thus, of similar age and body mass when sampled. The study obtained ethical clearance from the University of the Witwatersrand (AESC: 2007/38/04).

Data analysis

Data analysis was performed using the statistical software R (version 2.11.0 R Development Core Team 2006). Data were tested for normality using the Shapiro-Wilk Normality Test and are presented as mean \pm standard deviation. Exact p-value calculations were performed on all tests. We used generalized linear models (GLM) to compare: 1. group-living mice that remained so, with mice that would become solitary-living, before the latter dispersed; and 2. group-living and solitary-living individuals after the latter had dispersed and became solitary. The first GLM was used to test whether striped mice that would later become solitary already differed hormonally from striped

mice that remained group-living before dispersal. The second GLM tested whether group- and solitary-living individuals differed in hormone levels after dispersal. Each GLM had one of the hormones (corticosterone or testosterone) as the response variable; while sex or social category (group-living or solitary) were the fixed factors. We used linear mixed effect models (LMM) to compare: (i) striped mice that became solitary-living, before and after dispersal; and (ii) striped mice that remained group-living, before and after the dispersal of some of their group members. The first LMM was used to test whether striped mice change their hormone levels when they change social tactic. The second LMM was used to test whether hormone levels of group-living individuals might change following the departure of some of their group members even though their social tactic does not change. Each LMM had one of the two hormones as the response variable, sex and social category as the fixed factors and individual ID as a random factor to account for pseudo-replication. We selected the best model by a stepwise backward procedure (following Crawley, 2007). Hormone levels were square-rooted prior to analyses owing to their skewed distribution. We used LMMs and GLMs to compare hormone levels between subjects and paired t-tests to compare hormone levels within the same individuals before and after they became solitary.

RESULTS

Differences in hormone levels permanently group-living striped mice and group-living striped mice that later became solitary-living

Group-living striped mice males that would later become solitary displayed a trend towards having lower corticosterone levels than males that would remain group-living

(GLM: $F_{1,9} = 4.6, p = 0.06$; Fig. 1a). Group-living striped mice females that would later become solitary did not differ in their corticosterone levels from females that remained group-living (GLM: $F_{1,16} = 0.48, p = 0.50$; Fig. 1a). Dispersing males' corticosterone levels were significantly lower than those of dispersing females (GLM: $F_{1,9} = 6.73, p = 0.029$; Fig. 1a), whereas corticosterone levels of striped mice males and females that remained group-living did not differ (GLM: $F_{1,16} = 0.43, p = 0.52$; Fig. 1a).

Group-living striped mice males that would later become solitary did not differ in their testosterone levels from males that would remain group-living (GLM: $F_{1,7} = 0.33, p = 0.58$; Fig. 1b); and group-living striped mice females that would later become solitary did not differ in their testosterone levels from females that would remain group-living (GLM: $F_{1,11} = 2.43, p = 0.15$; Fig. 1b). Whereas dispersing males' testosterone levels did not differ significantly from those of dispersing females (GLM: $F_{1,8} = 0.13, p = 0.72$; Fig. 1b), non-dispersing males had higher testosterone levels than non-dispersing females (GLM: $F_{1,10} = 9.62, p = 0.01$; Fig. 1b).

Comparison of hormone levels between solitary individuals before and after they became solitary

We compared hormone levels of mice that were group-living at the start of the experiment but later became solitary (middle bars of Fig. 1) with hormone levels of solitary mice at the end of the experiment (right bars in Fig. 1). Striped mice that were group-living at the start of the experiment but later became solitary had significantly higher corticosterone levels than solitary-living striped mice (at the start of the experiment: 1437.85 ± 832.44 vs. at the end of the experiment: 575.62 ± 497.13 ; LMM:

$F_{1,5} = 16.73, p = 0.009$). Specifically, males that were group-living at the start of the experiment, but would later become solitary, had significantly higher corticosterone levels than solitary-living males (GLM: $F_{1,14} = 7.04, p = 0.02$; Fig. 1a); and females that were group-living at the start of the experiment, but would later become solitary, showed a trend towards having higher corticosterone levels than solitary-living females (LMM: $F_{1,3} = 7.55, p = 0.07$; Fig. 1a). Corticosterone levels of males were significantly lower than females both at the start (GLM: $F_{1,9} = 5.50, p = 0.04$; Fig. 1a) and at the end of the experiment (GLM: $F_{1,18} = 11.62, p = 0.003$; Fig. 1a).

Male striped mice that were group-living at the start of the experiment but later became solitary had significantly lower testosterone levels than solitary-living males (GLM: $F_{1,14} = 13.16, p = 0.003$; Fig. 1b). Testosterone levels of females that were group-living at the start of the experiment but later became solitary remained similar to those of solitary-living females (LMM: $F_{1,3} = 1.14, p = 0.36$; Fig. 1b). While testosterone levels of males that would become solitary did not differ from females that would become solitary at the start of the experiment (GLM: $F_{1,8} = 0.13, p = 0.72$; Fig. 1b), solitary-living males had significantly higher testosterone levels than solitary-living females (GLM: $F_{1,18} = 23.51, p < 0.0001$; Fig. 1b).

In addition, we compared changes in hormone levels for six individuals (four females and two males for corticosterone and three females and two males for testosterone) for which we had samples when they were still in the group and after they had become solitary (paired data). Striped mice that dispersed and adopted a solitary life significantly decreased their corticosterone levels (before: 1427.59 ± 1020.01 vs. after: 664.74 ± 681.68 ; $t = 2.28, df = 5, p = 0.04$). Testosterone levels increased considerably in

both males (male 1 increased from 0.33ng/ml to 5.55 ng/ml and male 2 increased from 0.80 ng/ml to 1.06 ng/ml), and in three out of four females (from 0.37 ± 0.41 to 1.03 ± 1.17).

Hormonal adjustment in group-living individuals following dispersal of group members

Corticosterone levels of both group-living striped mice males and females remained similar after the departure of some of their group members (males before: 1645.67 ± 756.25 vs. after: 1504.82 ± 780.39 ; GLM: $F_{1, 11} = 0.02$, $p = 0.89$; females before: 1426.00 ± 420.28 vs. after: 1382.29 ± 622.40 ; LMM: $F_{1, 3} = 0.11$, $p = 0.79$).

Testosterone levels of group-living females showed a trend towards increasing following the departure of some of their group-members (before: 0.19 ± 0.20 vs. after: 0.60 ± 0.52 ; GLM: $F_{1, 11} = 4.30$, $p = 0.06$), while testosterone levels of group-living males remained similar even after the departure of some of their group members (before: 0.77 ± 0.45 vs. after: 0.54 ± 0.48 ; GLM: $F_{1, 12} = 1.03$, $p = 0.33$).

Differences in hormone levels between individuals that remained group-living and individuals that became solitary after dispersal of the latter

Solitary striped mice males had significantly lower corticosterone levels than their conspecifics that remained group-living (solitary: 301.12 ± 304.22 vs. group-living: 1382.29 ± 622.40 ; GLM: $F_{1, 16} = 26.72$, $p < 0.0001$) and significantly higher testosterone levels (solitary: 3.85 ± 2.39 vs. group-living: 0.54 ± 0.48 ; GLM: $F_{1, 20} = 30.31$, $p < 0.0001$). Solitary females had significantly lower corticosterone than females that remained group-living (solitary: 791.11 ± 356.87 vs. group-living: 1504.82 ± 780.39 ;

GLM: $F_{1, 11} = 5.18, p = 0.04$), whereas they did not differ in their testosterone levels from group-living females (solitary: 0.63 ± 0.71 vs. group-living: 0.60 ± 0.52 ; GLM: $F_{1, 12} = 0.004, p = 0.95$).

DISCUSSION

Striped mice of both sexes that became solitary had significantly lower corticosterone levels than individuals that remained group-living, suggesting that adopting a solitary tactic can be a way to avoid social stress arising from group-living. Males that left their natal group to become solitary increased their testosterone levels but not males that remained group-living. Females that became solitary showed a trend to decrease their corticosterone levels, which, nonetheless, became significantly lower than their conspecifics that remained group-living, but did not show an increase in their testosterone levels. In a previous study, we demonstrated that males that dispersed were scrotal while males that remained philopatric in their natal group were unscrotal; and that 75% of females that dispersed, but only 13% of females that remained philopatric, were breeding (Schoepf and Schradin, 2012a). Our results show that group-living males and females differ considerably in their hormone profiles from solitary-living males and females and indicate that hormone levels change when striped mice switch from group- to solitary-living.

Glucocorticoids affect reproductive behavior by regulating the availability of energy by influencing glucogenesis, fat and protein metabolism (Romero, 2002; Reeder and Kramer, 2005). In some group-living birds and mammals, dominant individuals have higher glucocorticoid levels than subordinates, while in other species dominants have

lower levels. This difference might depend on whether it is more stressful to occupy a dominant or a subordinate rank (Creel, 2001). One suggestion is that dominant individuals suppress the reproduction of subordinates by inducing chronic stress, resulting in sustained high levels of glucocorticoids in subordinates, the “physiological castration” hypothesis (Abbott et al., 2003; Reyer et al. 1986). Our results taken together with results from previous studies (Schradin, 2008; Schradin and Yuen, 2011; Schradin et al., 2009b; Schradin et al., 2012b; Schradin et al., 2012c) indicate that this is the case in philopatric male striped mice. Chronically increased glucocorticoid levels indicate allostatic load, i.e. physiological costs due to an over activation of the neuroendocrine stress response (McEwen and Wingfield, 2003). As a consequence, philopatric males should leave their natal group when costs of dispersing to become solitary are lower than costs of remaining philopatric. By reducing population density we experimentally offered such option. In our study, corticosterone levels of solitary males only showed a trend towards decreasing before these individuals changed from group-living to solitary. While corticosterone levels of males became significantly much lower after individuals switched from a social to a solitary tactic. The difference in corticosterone levels between group-living and solitary individuals we found was much more marked than the difference between individuals that were initially group-living but later became solitary and individuals that remained group-living. Additionally, males that switched tactic were already sexually mature before dispersal (Schoepf and Schradin, 2012a), which is in agreement with the observation that high testosterone levels are not compatible with high corticosterone levels in this species (Raynaud et al., 2012).

After becoming solitary, females retained significantly higher corticosterone levels than males, indicating important physiological differences between the sexes. In mammals, breeding females need high glucocorticoid levels to deal with the energetic demands of lactation and pregnancy (Reeder and Kramer, 2005), and in striped mouse breeding females have high corticosterone levels (Schradin, 2008). Nevertheless, also in female striped mice high corticosterone levels could be an indicator of stress, including social stress, and our present study found that philopatric females that remained group-living had higher corticosterone levels than females that became solitary. Most importantly and, in contrast with philopatric males, philopatric females can breed in their natal group when population density is low (Schradin et al. 2010). In an accompanying study we showed that 75% of females that became solitary reproduced, but only 13% of females that remained group-living bred (Schoepf and Schradin, 2012a). Thus, increased corticosterone levels due to social stress could be one explanation why young philopatric females often do not breed, but this needs further investigation, particularly as our results showed that females that switched from group- to solitary-living displayed only a trend toward decreasing their corticosterone levels. The situation in females seems to be thus more complex than in males and further studies are needed to elucidate it.

Dispersal of individuals from a group might affect others that remain in the group. Dispersal of siblings has been shown to reduce competition over food and to increase body condition in screech-owls (*Otus asio* and *Otus kennicottii*; Belthoff and Dufty, 1998). In our study, dispersal of group-members did not cause changes in corticosterone levels in the individuals that remained group-living, suggesting that a decrease in group

size did not decrease stress levels, supporting the idea that remaining as a non-breeding helper within the group is stressful.

Group-living striped mice that later became solitary did not differ in their testosterone levels from striped mice that would remain group-living. Testosterone levels of philopatric males also remained similar before and after departure of some of their group-members, suggesting that reproductive suppression by their father rather than other factors, such as population density, is the cause of their retained low testosterone levels. Testosterone levels of solitary males, however, significantly increased. Similarly, previous research has shown that philopatric male striped mice that become solitary roaming males increase their testosterone levels and decrease their corticosterone levels (Schradin and Yuen, 2011). In males, testosterone has several functions, which among others include the regulation of aggression, sexual behavior and dispersal (Ketterson and Nolan, 1999; Wingfield et al., 2001; Nelson, 2005). Testosterone levels of solitary males in our study were comparable to testosterone levels measured in solitary males following a roaming tactic (Schradin et al., 2009a). Philopatric males normally have smaller testes and lower sperm counts (Schradin et al., 2009b), as well as lower testosterone and higher corticosterone levels than roaming males (Schradin et al., 2009a). Striped mice dispersed to seek independent breeding opportunities as soon as free territories were made experimentally available (Schoepf and Schradin, 2012a). While most philopatric males are sexually suppressed, males that dispersed were already sexually mature (i.e. scrotal) at the time of dispersal (Schoepf and Schradin, 2012a). Thus, physiological changes might have been initiated already shortly before dispersal. However, our current study demonstrates that these physiological changes did not occur before we manipulated the

environment and made territories available. Thus, it is likely that an increase of testosterone levels shortly before dispersal might trigger the switch from group- to solitary-living.

Testosterone levels of females were very similar to testosterone levels of philopatric males, independently of their dispersal status, and comparable to those shown in a previous study (Schradin 2008). In contrast to males, female testosterone levels did not increase significantly after dispersal and staid in a low but measurable range. One of the many functions of testosterone is to promote sexual motivation in males (Ketterson and Nolan, 1999; Wingfield et al., 1990), while its functions in female vertebrates are poorly understood. Testosterone in female mammals may also, as it is in male, be associated with dispersal (Holekamp et al., 1984; Nunes et al., 1999), and there are increasing evidence, at least in some species, that in the absence of reproductive suppression testosterone levels can become higher even in females (Lutermann et al., in press). In our study, females that dispersed and became solitary had nearly double as high testosterone levels as philopatric females, but this difference was not significant and overall testosterone levels of solitary females were low. The lower testosterone levels in females as compared to males might indicate that in female striped mice testosterone is not as important in reproduction as it is for males. Like in males, females that became solitary started breeding, while most females that remained philopatric did not (Schoepf and Schradin, 2012a), providing evidence that other hormones rather than testosterone might play an important role in dispersal (perhaps progesterone or estrogen which were not studied here, due to the small amounts of serum available). Interestingly, females' testosterone levels increased following the departure of some of their group-members, but

393 did not become higher than those of females that became solitary-living, and, at the
394 moment, it is not clear whether the significant increase we observed has any biological
395 significance, especially as testosterone levels were still relatively low.

396 Our results revealed important insights into the physiological mechanisms
397 underlying the differences between group-living and solitary individuals. Dispersing
398 males had lower corticosterone levels than philopatric males. As breeding males need low
399 corticosterone levels to allow for high testosterone levels necessary for spermatogenesis
400 (Raynaud et al., 2012), this might indicate that these males were already physiologically
401 primed to disperse and start independent breeding. Corticosterone levels were much
402 lower in solitary striped mice of both sexes. High corticosterone levels indicate high
403 physiological and metabolic costs for group-living individuals. Solitary males had
404 significantly higher testosterone levels than group-living males, suggesting that they were
405 no longer reproductively suppressed. Ours is one of very few studies comparing hormone
406 levels between alternative social and reproductive tactics in both sexes. Taken together
407 with previously published results (see Schoepf and Schradin, 2012a, 2012b) our findings
408 indicate a link between environmental change, behavioral adaptation and underlining
409 physiological mechanisms, which result in significant fitness consequences.

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584

585 **TABLES AND FIGURES**

586 **Table 1.**

587 Total number of individuals sampled for corticosterone and testosterone throughout the
588 study. “Philopatric” indicate individuals that remained permanently group-living and
589 “Solitary” individuals that were originally group-living, but later became solitary.
590 Numbers in parenthesis indicate individuals that were sampled twice.

591

592 **Figure 1.**

593 Comparison of hormone levels between permanently group-living mice (labeled as
594 ‘*Group-living*’), group-living mice that later became solitary (labeled as ‘*Group-living* →
595 *Solitary*’), and, solitary-living mice (labeled as ‘*Solitary*’). a) Corticosterone and b)
596 Testosterone. $p^* < 0.05$; $p^{**} < 0.01$; $p^{***} < 0.001$

597

598 **Table 1.**

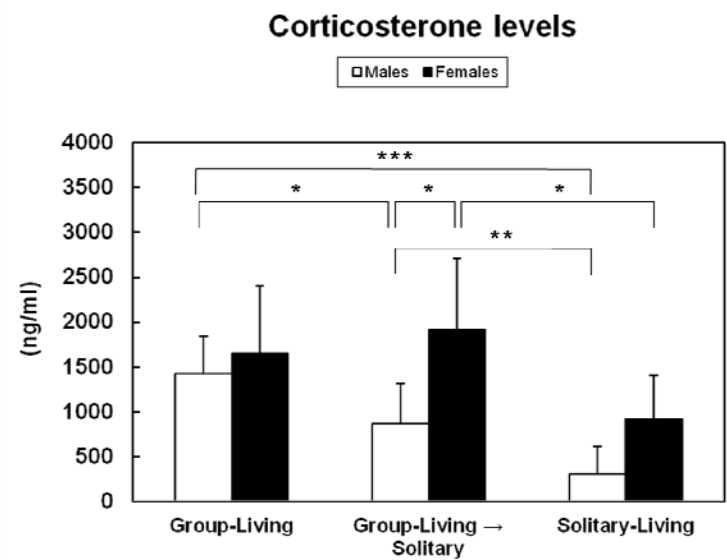
		Before	After
Corticosterone	Philopatric	<i>18</i>	<i>12</i>
	Solitary	<i>11 (6)</i>	<i>19 (6)</i>
Testosterone	Philopatric	<i>12</i>	<i>15</i>
	Solitary	<i>10 (5)</i>	<i>20 (5)</i>

599

600

Figure 1.

a)



b)

